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| <u>L20</u> | (DNA or RNA or Nucleic acid) near5 immobiliz\$ near5 (divalent or magnesium) near5 EDTA | 0 | <u>L20</u> |
| <u>L19</u> | l18 and (purifY or isolat\$ or extract\$) | 8 | <u>L19</u> |
| <u>L18</u> | L17 and single stranded nucleic acid | 9 | <u>L18</u> |
| <u>L17</u> | L16 and EDTA | 45 | <u>L17</u> |
| <u>L16</u> | (DNA or RNA or nucleic acid) near5 (immobiliz\$ or bind\$) near5 (divalent or magnesium) | 68 | <u>L16</u> |
| <u>L15</u> | (DNA or RNA or nucleic acid) near5 (immobiliz\$ or bind\$) | 25086 | <u>L15</u> |
| <u>L14</u> | (DNA or RNA or nucleic acid) neaa5 (immobiliz\$ or bind\$) | 0 | <u>L14</u> |
| <u>L13</u> | L1 and (divalent near5 magnesium near5 chelat\$) | 15 | <u>L13</u> |
| <u>L12</u> | l1 and (immobiliz\$ near5 magnesium near5 chelat\$) | 0 | <u>L12</u> |
| <u>L11</u> | l1 and immobiliz\$ | 10496 | <u>L11</u> |
| <u>L10</u> | L9 and magnesium | 0 | <u>L10</u> |
| <u>L9</u> | (purif\$ or isolat\$ or extract\$) near5 (DNA or RNA or nucleic acid) near5 divalent | 11 | <u>L9</u> |
| <u>L8</u> | L7 and guanidinium | 107 | <u>L8</u> |
| <u>L7</u> | l3 and magnesium | 1006 | <u>L7</u> |
| <u>L6</u> | L5 and guanidinium | 24 | <u>L6</u> |
| <u>L5</u> | L4 and chaotropic | 92 | <u>L5</u> |
| <u>L4</u> | L3 and Mg | 1594 | <u>L4</u> |
| <u>L3</u> | l1 and (divalent or MGg) | 2527 | <u>L3</u> |
| <u>L2</u> | L1 and divalent positive | 0 | <u>L2</u> |
| <u>L1</u> | (purif\$ or isolat\$ or extract\$) near5 (DNA or RNA or nucleic acid) | 47348 | <u>L1</u> |

END OF SEARCH HISTORY

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11. 5756126. 07 Jun 95; 26 May 98. Dry solid medium for storage and analysis of genetic material. Burgoyne; Leigh Alexander. 424/488; 422/55 422/56 422/57 435/174 435/183 435/4 435/5 435/6 435/7.1 435/7.2 435/7.9 435/91.2 435/970. A61K009/14 C12Q001/68 C12Q001/70 C07H021/04.

12. 5747290. 06 Jun 95; 05 May 98. Process for the production of recombinant polypeptides. Emtage; John Spencer, et al. 435/69.4; 435/320.1 536/23.51. C12P021/02 C07H021/04 C12N015/70.

13. 5639599. 06 Jun 94; 17 Jun 97. Amplification of nucleic acids from mononuclear cells using iron complexing and other agents. Ryder; Thomas B., et al. 435/5; 435/6 435/91.2 435/91.21. C12Q001/70 C12Q001/68 C12P019/34.

14. 5496562. 30 Nov 93; 05 Mar 96. Solid medium and method for DNA storage. Burgoyne; Leigh A.. 424/488; 424/443 424/464. A61K009/14 A61K009/70.

15. 5482836. 14 Jan 93; 09 Jan 96. DNA purification by triplex-affinity capture and affinity capture electrophoresis. Cantor; Charles R., et al. 435/6; 204/456 435/91.1 536/23.1 536/24.3 536/24.33 536/25.3 536/25.32. C12Q001/68 C12P019/34 C07H017/00 C12N015/00.

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| Term | Documents |
|---|-----------|
| DIVALENT.DWPI,EPAB,JPAB,USPT. | 96170 |
| DIVALENTS.DWPI,EPAB,JPAB,USPT. | 40 |
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| MAGNESIUMS.DWPI,EPAB,JPAB,USPT. | 147 |
| MAGNEZIA.DWPI,EPAB,JPAB,USPT. | 28174 |
| MAGNEZIAS.DWPI,EPAB,JPAB,USPT. | 226 |
| CHELAT\$ | 0 |
| CHELAT.DWPI,EPAB,JPAB,USPT. | 33 |
| CHELABILITY.DWPI,EPAB,JPAB,USPT. | 30 |
| CHELATABL.E.DWPI,EPAB,JPAB,USPT. | 290 |
| CHELATABLES.DWPI,EPAB,JPAB,USPT. | 1 |
| (L1 AND (DIVALENT NEAR5 MAGNESIUM NEAR5 CHELAT\$)).USPT,JPAB,EPAB,DWPI. | 15 |

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L13: Entry 13 of 15

File: USPT

Jun 17, 1997

DOCUMENT-IDENTIFIER: US 5639599 A

TITLE: Amplification of nucleic acids from mononuclear cells using iron complexing and other agents

Brief Summary Text (3):

It is common to require nucleic acid to be isolated and purified (i.e., prepared) from various tissues in order to detect the presence of a particular nucleic acid--for example, the presence of HIV-1 DNA or RNA in a blood cell of a human. For this purpose, the nucleic acid is generally extracted after extensive purification of appropriate blood cells, lysis of these cells and purification of the released nucleic acids to remove substances that might inhibit later analytical procedures. In particular, it is important to produce nucleic acid of a quality and purity to allow its amplification.

Brief Summary Text (6):

The present invention is directed to methods and kits for the preparation of nucleic acid, and particularly for isolation of DNA or RNA from cells, such as mononuclear cells (e.g., T-lymphocytes and/or monocytes), for amplification of that nucleic acid. Such amplified nucleic acid may then be used for various purposes, including screening the nucleic acid for the presence of viral nucleic acid sequences, using a probe which is complementary to a selected nucleic acid sequence of the virus. It is also useful for detection of genetic anomalies or defects in the nucleic acid. Accordingly, the methods and kits are designed to allow rapid and easy preparation of nucleic acid without the need for extensive purification procedures.

Brief Summary Text (7):

Thus, in a first aspect, the present invention features a method for preparing nucleic acid from cells for amplification. A sample containing various cells (e.g., whole blood) and an appropriate centrifugation medium are centrifuged to cause a population of one cell type to gather in a discrete layer. This layer is separate and distinct from the remainder of the cells and detritus in the sample, except for a small amount of platelets and/or lipids or other low density components, or other soluble and suspended constituents. Surprisingly, the presence of platelets and other components does not prevent amplification of nucleic acid purified in this method.

Detailed Description Text (2):

The claimed method features a series of steps for the collection, isolation, preparation, amplification and screening of nucleic acid, preferably from mononuclear cells, and a combination of apparatus, media and agents to effectuate such a method. The various steps, apparatus, media and agents are discussed generally above, and examples are now provided.

Detailed Description Text (20):

After removing the isolated desired cells, the cells are lysed to release the nucleic acid from, or associated with, the cells. This means any nucleic acid either attached to or found within or on the desired cells.

Detailed Description Text (25):

Examples of chelating agents that may be used are deferoxamine and transferrin. Deferoxamine has a 10.^{sup.31} binding constant for the ferric ion, see Antioxidant Capacity of Desferrioxamine and Ferrioxamine in the Chemically-Initiated Lipid Peroxidation of Rat Erythrocyte Ghost Membranes, Videla, C. A., et al., Biochem. Int'l, 16, 799 (May 1988), and binds preferentially to zinc and ferric ions magnesium ions. Deferoxamine has a 10.^{sup.11.1} binding constant for Zn.^{sup.2+}, and a 10.^{sup.4.3} binding constant for Mg.^{sup.2+}. Examples of calcium ion complexing agents are oxalate and citrate. Oxalate and citrate also bind Fe.^{sup.+++} with greater stability compared

to Mg^{sup.++}, especially at neutral-alkaline pH. Other agents, such as EDTA, are not preferred chelating agents, as they bind zinc ion and magnesium ion, along with other divalent cations.

Detailed Description Text (70):

The above lysate contains a crude mixture of biologically derived molecules, many of which are low molecular weight hydrolysis products of the original sample constituents. The DNA present in these lysates, while fragmented and denatured compared to its original structure within the cells, is still a suitable reactant for a variety of chemical and biochemical reactions, including as a template for nucleic acid target amplification. To minimize the complexity and manipulations involved in practical, routine use of target amplification to analyze blood-derived nucleic acids, in one aspect of the invention the lysate is added directly to the amplification reaction as the source of the analyte, without further purification. However, it is well known that biologically-derived samples frequently contain components that are inhibitory to in vitro biochemical reactions, including enzyme reactions. For example, even nucleic acid preparations that are much more highly purified than the lysate yielded by the method described herein have been reported to contain potent inhibitors of polymerase chain reaction amplification (R. deFranchis, N. C. P. Cross, N. S. Foulkes and T. M. Cox, A Potent Inhibitor of Taq Polymerase Copurifies with Human DNA, Nucleic Acids Research 16:10355 (1988)).

Detailed Description Text (85):

3.) Add to this mixture, 40 .mu.l of the hydrolyzed MNC suspension prepared as described above. Optionally add a known amount of purified nucleic acid containing the target sequence(s) of interest as a basis for comparing amplification performance supported by different conditions.

Other Reference Publication (34):

Walsh, et al., "Chelex.RTM. 100 as a Medium for Simple Extraction of DNA for PCR-Based Typing from Forensic Material," Biotechniques 10:506-513 (1991).

End of Result Set

L13: Entry 15 of 15

File: USPT

Jan 9, 1996

DOCUMENT-IDENTIFIER: US 5482836 A

TITLE: DNA purification by triplex-affinity capture and affinity capture electrophoresisAbstract Text (1):

The invention provides a method for purifying or isolating double stranded DNA intact using triple helix formation. The method includes the steps of complexing an oligonucleotide and double stranded DNA to generate a triple helix and immobilization of the triple helix on a solid phase by means of a molecular recognition system such as avidin/biotin. The purified DNA is then recovered intact by treating the solid phase with a reagent that breaks the bonds between the oligonucleotide and the intact double stranded DNA while not affecting the Watson-Crick base pairs of the double helix. The present invention also provides a method for purifying or isolating double stranded DNA intact by complexing the double stranded DNA with a specific binding partner and recovering the complex during electrophoresis by immobilizing it on a solid phase trap imbedded in an electrophoretic gel.

Brief Summary Text (2):

The present invention relates generally to a method for purifying or isolating double stranded DNA using triple-helix formation and solid phase separation. It also relates to a triple-helix immobilized on a solid phase by means of a molecular recognition system such as avidin/biotin. The present invention further relates to a method for purifying or isolating double stranded DNA by complexing the DNA with a specific binding partner and immobilizing the complex during electrophoresis on a solid phase trap embedded in the electrophoretic gel. The invention still further relates to agarose gels containing a trap comprising a solid phase directly or indirectly fixed with recognition molecules from a molecular recognition system.

Brief Summary Text (6):

Typically, specific DNA is isolated from heterogeneous DNA mixtures using conventional hybridization based methods (e.g., colony or plaque hybridization (Sambrook, J., et al. (1989) Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor, Cold Spring Harbor Press)). These well-established methods while quite reliable have some practical drawbacks. First, they require time-consuming and labor-intensive steps of filter preparation that often limit the number of clones that can be screened. Furthermore, since these procedures include prior denaturation steps and other treatments that destroy the integrity of the target DNA molecules, one has to reisolate the corresponding clones from the original plates to obtain intact DNA molecules for further biological biochemical manipulations. Third, sequences toxic to the host sometimes hamper successful cloning. Fourth, the natural modifications of the target DNA are not maintained during cloning. Finally, despite recent development of Yeast Artificial Chromosome (YAC) vectors, it is still difficult to clone very large DNAs. Obviously, non-cloning-based biochemical methods to isolate specific DNA from a complex mixture would be of some help with these problems. However, such methods are still not satisfactory. Biochemical purification by density and size fractionation after cleavage with restriction enzymes can be applied only in limited instances (Tsujimoto, Y. and Suzuki, Y. (1984) Proc. Natl. Acad. Sci. USA 81:1644-1648). The polymerase chain reaction (PCR) fulfills some of these needs and provides large amounts of DNAs (Mullis, K. B. and Falocna, F. A. (1987) Methods Enzymol 155:335-350), but it is currently limited to relatively short (<10 kb) DNA fragments. Furthermore, natural modifications of the original DNA cannot be maintained. Although an effective method of affinity chromatography for DNAs was reported (Tsurui, H. et al. (1990) Gene 88:233-239), it requires the prior denaturation of target DNA molecules and elution by denaturation.

Brief Summary Text (10):

The present invention is a method for isolating double stranded DNA in a sample using triple-helix formation and solid phase separation. The sample is contacted with an oligonucleotide coupled either directly or indirectly to a first recognition molecule of a specific molecular recognition system. A molecular recognition system is a system of at least two molecules which have a high capacity of molecular recognition for each other and a high capacity to specifically bind to each other. The coupled oligonucleotide forms a triple-helix with the particular target DNA by means of Hoogsteen hydrogen bonding. The reaction medium containing the triple-helix is then contacted with a solid carrier to which is either directly or indirectly fixed a second recognition molecule of the molecular recognition system causing the second recognition molecule to specifically bind to the first recognition molecule. The solid phase bearing the triple-helix is then separated from the reaction medium in which the binding between the recognition molecules occurred and the particular target DNA is separated from the oligonucleotide by treating the separated solid phase with a reagent that breaks the bonds between the oligonucleotide and the particular DNA but not between the double helix DNA. The intact particular DNA is then recovered.

Brief Summary Text (13):

The present invention further provides a method for isolating a particular intact double stranded DNA in a sample which comprises the steps of incubating a sample containing the particular DNA with a binding partner specific for the DNA for a time sufficient for the binding partner to bind to the particular DNA. The specific binding partner is coupled either directly or indirectly to a first recognition molecule of a specific molecular recognition system. The reaction mixture containing the DNA bound to the coupled binding partner is then electrophoresed in a gel containing a gel-embedded solid phase to which is either directly or indirectly fixed a second recognition molecule of the molecular recognition system. During electrophoresis, the second recognition molecule specifically binds to the first recognition molecule coupled to the specific binding partner bound to the particular DNA. Following electrophoresis, the solid phase is separated from the gel and the particular DNA is separated from the solid phase by treating the solid phase with a reaction medium that breaks the bonds between the DNA and the specific binding partner but not between the double stranded target DNA. The DNA is then recovered.

Drawing Description Text (4):

FIG. 3A represents a photograph of an electrophoretic agarose gel showing the results of a PCR-based assay for $(dT-dC) \cdot sub.n - (dG-dA) \cdot sub.n$ sequences on clones purified by the triplex-affinity capture method of the invention from a chromosome 21 specific library. The gel contains DNA from purified clones 1 and 3 and digested .lambda. DNA following electrophoresis.

Drawing Description Text (5):

FIG. 3B represents a photograph of an electrophoretic agarose gel showing the results of a PCR-based assay for $(dT-dC) \cdot sub.n - (dG-dA) \cdot sub.n$ sequences on clones purified by the triplex-affinity capture method of the invention from a chromosome 21 specific library. The gel contains DNA from purified clones 4-6 and digested .lambda. DNA following electrophoresis.

Detailed Description Text (2):

This invention is directed to a method for purifying intact DNA using intermolecular triple-helix formation and solid phase separation. In this triplex-affinity capture (TAC) method, the DNA being detected in the assay is intact double stranded DNA and the method can be used to capture sequence specific plasmid DNAs. Essentially, the target DNA sequence is a double stranded homopurine-homopyrimidine helix. Nevertheless, the method may be extended by the use of some permissiveness mismatches in triple-helix formation (Griffin, L. C., et al. (1989) Science 245:967-971 and Belotserkovskii, B. D., et al. (1990) Nucleic Acids Res. 18:6621-6624), alternate strand triple-helix formation (Horne, D. A., et al. (1990) J. M. Chem. Soc. 112:2435-2438), other types of triple-helices (Cooney, M., et al. (1988) Science 241:456-459; Kohwi, Y., et al. (1988) Proc. Natl. Acad. Sci. USA 85:3781-3785; Letai, A. G., et al. (1988) Biochemistry 27:9108-9112; Bernues, J., et al. (1989) EMBO J. 8:2087-2094; Beal, P. A., et al. (1991) Science 251:1360-1363; Pilch, D. S., et al. (1991) Biochemistry 30:6081-6087; Orson, F. M., et al. (1991) Nucleic Acids Res.

19:3435-3441), including ones formed by recombinase proteins (Hsieh, P., et al. (1990) Genes Dev. 4:1951-1963; Rao, B. J., et al. (1991) Proc. Natl. Acad. Sci. USA 88:2984-2988) and artificial base analogs.

Detailed Description Text (4):

The TAC method for purifying a particular double strand of DNA comprises contacting the sample with an oligonucleotide coupled either directly or indirectly to a first recognition molecule of a specific molecular recognition system. The oligonucleotide is designed to specifically form a triple helix with the target DNA. Methods for designing such oligonucleotides depend on the target DNA. Acceptable methods are set forth in Kiessling, L. L., et al. (1992) Biochemistry 31:2829-2834; Durland, R. H., et al. (1991) Biochemistry 30:9246-9255; Beal, P. A., et al. (1992) Nucleic Acids Res. 20:2773-2776; Giovannangeli, C., et al. (1992) PNAS 89:8631-8635; Beal, P. A., et al., (1992) J. Am. Chem. Soc. 114:4976-4982. Oligonucleotides which contain deoxyuracil for thymine at least along part of the chain are acceptable oligonucleotides.

Oligonucleotide backbone analogs such as polyamide nucleic acids and phosphotriesters will form a triplex with double stranded DNA and can also be used in the TAC method of the invention.

Detailed Description Text (12):

For use in the present invention, the oligonucleotide is preferably a homopyrimidine oligonucleotide (T-C).sub.n. But a purine-rich oligonucleotide which binds to double stranded DNA at neutral pH in the presence of multivalent cations (e.g., spermine or Mg.sup.2+) to form a second type of triplex (Cooney, M., et al. (1988) Science 241:456-459; Beal, P. A., et al. (1991) Science 251:1360-1363) may also be used. After solid phase separation of this type of triplex by using a specific molecular recognition system, the triplex bond can be broken by incubating the solid phase containing the triplex in a buffer containing EDTA or any other magnesium chelate to chelate divalent cations and release the target DNA. This type of triplex is not affected by the non-specific interaction between DNA and streptavidin and therefore enables the use of fairly non problematic electrophoretic buffers in the electrophoretic capture aspects of the invention.

Detailed Description Text (16):

Following incubation and the immobilization of the triplex on the solid phase following either binding of the first recognition molecule to the second recognition molecule or following triplex formation, the solid phase bearing the triplex is separated from the reaction medium. The method of separation will depend upon the type of solid carrier used. Separation can occur by draining the reaction medium off of and away from the solid phase and or washing the solid phase. When the solid carrier is the preferred magnetic beads, the beads can be separated by a magnetic particle concentrator and washed with buffer (see, Uhlen, M. (1989) Nature (London) 340:733-734). Finally, the solid phase is incubated with a reagent which will break the Hoogsteen hydrogen bonds between the DNA and the oligonucleotide. Preferably, the reagent is a basic buffer with a pH ranging from about 8.0 to about 13.0. More preferably, from about 8.0 to about 10.0. Most preferably, 1.0M Tris.multidot.HCl, in 0.5 MEDTA at a pH of about 9. However, other buffers such as TE (10 mM Tris-HCl (pH 8), 1 mM EDTA are also acceptable. Again, incubation time will depend upon the choice of the reagent. Care should be taken that the reagent used to break the bonds between the DNA and the oligonucleotide breaks only the Hoogsteen hydrogen bonds and not the Watson and Crick double helical DNA bonds. Triplexes using oligonucleotide backbone analogs can be formed and broken under conditions or reagents similar to those used for regular oligonucleotides. Following separation of the target DNA from the oligonucleotide, the intact target double stranded DNA is recovered from the eluate by conventional means such as phenyl/chloroform extraction (1:1 (vol/vol) and ethanol precipitation or electrophoresis.

Detailed Description Text (18):

This invention is further directed to a method for purifying sequence specific DNA and large intact DNA (i.e., greater than 100,000 base pairs) by binding the particular DNA to a specific binding partner coupled to a molecular recognition molecule and immobilizing the bound DNA during electrophoresis on a solid phase trap bearing a second recognition molecule wherein the trap is embedded in the electrophoretic gel. This affinity capture electrophoresis (ACE) method selectively captures the large target DNA molecule bound to its specific binding partner while the other non-target

molecules pass through the trap.

Detailed Description Text (20):

In another of the preferred embodiments, the target DNA comprises DNA fragments the ends of which have been converted to single stranded DNA. However, this method can also isolate any DNAs if RecA or similar recombinase proteins are used to assist triple helix formation between the probe and the target DNA sequence. (Ferrin, L. J., et al., (1991) Science 254:1494-1497. In the ACE method of the invention, the bound target DNA is attached to a solid phase which is too large to migrate through the gel pores during electrophoresis. The preferred gel material is agarose but polyacrylamide, other synthetic polymer gels or any convective but hydrophilic medium can also be used. The targeted DNA is selectively trapped while the other non-target molecules pass through the trap. The trap is then separated from the gel. After separation, the trap is treated with appropriate reagents which destroy the bonds between the coupled specific binding partner probe and the desired DNA. This treatment releases the target DNA and allows purification of the intact DNA.

Detailed Description Text (22):

In another preferred embodiment of the affinity capture electrophoresis method of this invention, DNAs which do not contain homopurine/homopyrimidine tracts can be isolated by using their ends as targets. Oligonucleotides using deoxyuracil for thymine and ribooligonucleotides are especially suited for "end capture" ACE.

Detailed Description Text (51):

Plasmid DNA (.about.2 .mu.g) from the library was isolated, digested with NotI and incubated with 10 pmol of 5'-biotinylated CT.sub.2 CT.sub.4 CT.sub.2 CT.sub.3 CT.sub.5 CT.sub.2 (SEQ ID NO:4) in 2M NaCl, 0.1M sodium phosphate buffer (pH 6.0) for 2 hr at 50.degree. C. Then, .about.0.2 mg of streptavidin-coated magnetic beads (Dynal, Streptavidin M-280) were added, collected by a magnet after 1 hour of incubation and washed extensively with the same buffer. Captured DNAs were released by incubation in 1M Tris-HCl (pH 9), 0.5 mM EDTA for 20 min, recovered by ethanol precipitation, recircularized and used for transformation of competent DH5.alpha. cells as set forth in Example 1. Plasmid DNA isolated from the cultures was analyzed by gel electrophoresis as also set forth in Example 1. One purification cycle at pH 6.0 yielded an enriched library with a complex electrophoretic pattern, although some bands were significantly enriched compared to the original library. A second TAC cycle at more stringent condition (pH 6.2) provided a simple population composed of only four DNA species, named A-D according to their sizes. Analysis of twelve randomly chosen clones showed that they contained two A, six B, two C and two D. Restriction mapping and PCR using primers specific to the LEU2 locus of this strain showed that clone B was the desired target. Other clones (A, C and D) may be derived from pseudo-target sites in this strain, as suggested by previous affinity cleaving experiments (Strobel, S. A. and Dervan, P. B. (1991) Nature 310:172-174). An additional cycle of TAC where all steps were performed at 50.degree. C. distinguished the target clone from the pseudo-clones, although the yield was low. Conditions for a single step purification of B from the total library were not identified.

CLAIMS:

1. A method for purifying intact a particular double stranded DNA present in a sample comprising the steps of:

(a) contacting the sample with an oligonucleotide coupled either directly or indirectly to a first recognition molecule of a specific molecular recognition system to form a triple-helix between the particular double stranded DNA and the coupled oligonucleotide, said oligonucleotide being an oligodeoxyribonucleotide or an oligoribonucleotide;

(b) contacting the reaction medium obtained in step (a) with a solid carrier to which is either directly or indirectly fixed a second recognition molecule of the molecular recognition system, the second recognition molecule specifically binding to the first recognition molecule;

(c) separating the reaction medium from the solid phase in step (b);

(d) separating the particular double stranded DNA from the oligonucleotide by treating the separated solid phase of step (c) with an alkaline reagent that breaks the bonds between the oligonucleotide and the particular double stranded DNA but conserves the double strandedness of the particular double stranded DNA, said reagent having a pH that is about 8.0 to about 10.0 if said oligonucleotide is an oligodeoxyribonucleotide or a pH that is no greater than about 8.5 if said oligonucleotide is an oligoribonucleotide; and

(e) recovering the particular double stranded DNA.

18. A method for purifying intact a particular double stranded DNA in a sample comprising the steps of:

(a) contacting the sample with a biotinylated oligonucleotide under acidic conditions to form by means of Hoogsteen hydrogen bonding, a triple-helix between the particular DNA and the oligonucleotide, said oligonucleotide being an oligodeoxyribonucleotide or an oligoribonucleotide;

(b) contacting the reaction medium obtained in step (a) with streptavidin coated magnetic beads to indirectly attach the triple-helix to the magnetic beads by means of biotin/streptavidin binding;

(c) separating the reaction medium from the magnetic beads;

(d) separating the particular double-stranded DNA from the oligonucleotide by incubating the magnetic beads of step (c) with a basic buffer that destabilizes the Hoogsteen hydrogen bonds between the oligonucleotide and the particular double-stranded DNA but not the Watson-Crick bonds, said buffer having a pH that is about 8.0 to about 10.0 if said oligonucleotide is an oligodeoxyribonucleotide or a pH that is no greater than about 8.5, if said oligonucleotide is an oligoribonucleotide; and

(e) recovering the particular double stranded DNA from step (d).

20. A method for isolating intact a particular double stranded DNA in a sample comprising the steps of:

(a) incubating a sample containing the DNA with an oligonucleotide for a time sufficient for the oligonucleotide to form a triple helix with the particular DNA, said oligonucleotide being coupled either directly or indirectly to a first recognition molecule of a specific molecular recognition system, wherein said oligonucleotide is an oligodeoxyribonucleotide or an oligoribonucleotide;

(b) electrophoresing the reaction mixture obtained in step (a) in a gel containing a gel embedded solid carrier to which is either directly or indirectly fixed a second recognition molecule of the molecular recognition system, the second recognition molecule specifically binding to the first recognition molecule in the reaction mixture during electrophoresis;

(c) separating the solid phase in step (b) from the gel following electrophoresis;

(d) separating the particular double stranded DNA from the solid phase by treating the solid phase from (c) with a reagent that breaks the bonds between the DNA and the oligonucleotide but conserves the double strandedness of the particular double stranded DNA, said reagent having a pH that is about 8.0 to about 10.0 if said oligonucleotide is an oligodeoxyribonucleotide or a pH that is no greater than about 8.5 if said oligonucleotide is an oligoribonucleotide; and

(e) recovering the particular double stranded DNA intact.

22. A method for isolating intact a particular double stranded DNA in a sample comprising the steps of:

(a) incubating a sample containing the particular DNA with a biotinylated homopyrimidine under acidic conditions to form by means of Hoogsteen hydrogen bonding

a triple-helix between the DNA and the homopyrimidine, said homopyrimidine being an oligodeoxyribonucleotide or an oligoribonucleotide;

(b) electrophoresing the reaction mixture obtained in step (a) under acidic conditions in a gel containing a trap comprising immobilized streptavidin coated beads embedded in said gel, whereby the biotin coupled to the oligonucleotide-DNA triple-helix of step (a) binds to the streptavidin coated beads during electrophoresis;

(c) separating the resulting solid phase in step (b) from the gel following electrophoresis;

(d) separating the particular double stranded DNA from the solid phase by treating the solid phase from (c) with a basic buffer to break the Hoogsteen hydrogen bonds between the DNA and the oligonucleotide but not the Watson-Crick bonds between the double-stranded DNA, said buffer having a pH that is about 8.0 to about 10.0 if said homopyrimidine is an oligodeoxyribonucleotide or a pH that is no greater than about 8.5 if said homopyrimidine is an oligoribonucleotide; and

(e) recovering the particular double stranded DNA intact.

24. A method for purifying intact a particular double stranded DNA in a sample comprising the steps of:

(a) contacting the sample with an oligonucleotide coupled either directly or indirectly to a first recognition molecule of a specific molecular recognition system and with a solid carrier to which is either directly or indirectly fixed a second recognition molecule of the molecular recognition system under conditions and for a time sufficient for the second recognition molecule to specifically bind to the first recognition molecule and for the coupled oligonucleotide and the DNA to form a triple helix, said oligonucleotide being an oligodeoxyribonucleotide or an oligoribonucleotide;

(b) separating the reaction medium from the solid phase in step (a);

(c) separating the particular double stranded DNA from the oligonucleotide by treating the separated solid phase of step (b) with an alkaline reagent that breaks the bonds between the oligonucleotide and the particular double stranded DNA but conserves the double strandedness of the particular double stranded DNA, said reagent having a pH that is about 8.0 to about 10.0 if said oligonucleotide is an oligodeoxyribonucleotide or a pH that is no greater than about 8.5 if said oligonucleotide is an oligoribonucleotide; and

(d) recovering intact the particular double stranded DNA.

25. A method for purifying intact a particular double stranded DNA in a sample comprising the steps of:

(a) contacting the sample with a specific oligonucleotide backbone analog coupled either directly or indirectly to a first recognition molecule of a specific molecular recognition system to form a triple-helix between the particular DNA and the coupled oligonucleotide analog, said oligonucleotide being an oligodeoxyribonucleotide or an oligoribonucleotide;

(b) contacting the reaction medium obtained in step (a) with a solid carrier to which is either directly or indirectly fixed a second recognition molecule of the molecular recognition system, the second recognition molecule specifically binding to the first recognition molecule;

(c) separating the reaction medium from the solid phase in step (b);

(d) separating the particular double stranded DNA from the oligonucleotide analog by treating the separated solid phase of step (c) with a reagent that breaks the bonds between the oligonucleotide backbone analog and the particular DNA but conserves the double strandedness of the particular double stranded DNA, said reagent having a pH that is about 8.0 to about 10.0 if said oligonucleotide is an oligodeoxyribonucleotide

or a pH that is no greater than about 8.5 if said oligonucleotide is an oligoribonucleotide; and

(e) recovering the particular double stranded DNA intact.

27. A method for purifying intact a particular double stranded DNA in a sample comprising the steps of:

(a) forming an immobilized triple helix with the particular DNA by means of a biotinylated oligonucleotide and streptavidin coated beads, wherein said oligonucleotide is an oligodeoxyribonucleotide or an oligoribonucleotide;

(b) separating the particular double stranded DNA from the triple helix in (a) by treating the triple helix with an alkaline reagent that breaks Hoogsteen hydrogen triplex bonds but not Watson-Crick hydrogen duplex bonds, said reagent having a pH that is about 8.0 to about 10.0 if said oligonucleotide is an oligodeoxyribonucleotide or a pH that is no greater than about 8.5 if said oligonucleotide is an oligoribonucleotide; and

(c) recovering the particular double stranded DNA intact.

FILE 'HOME' ENTERED AT 14:19:15 ON 13 SEP 2002

=> file medline caplus biosis
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SINCE FILE ENTRY TOTAL
FULL ESTIMATED COST 0.21 SESSION 0.21

FILE 'MEDLINE' ENTERED AT 14:19:50 ON 13 SEP 2002

FILE 'CAPLUS' ENTERED AT 14:19:50 ON 13 SEP 2002
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FILE 'BIOSIS' ENTERED AT 14:19:50 ON 13 SEP 2002
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=> (DNA or RNA or nucleic acid) (10a) (immobiliz? or attach?) (10a) (divalent or magnesium)

(DNA IS NOT A RECOGNIZED COMMAND

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For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (>).

=> s (DNA or RNA or nucleic acid) (10a) (immobiliz? or attach?) (10a) (divalent or magnesium)

L1 21 (DNA OR RNA OR NUCLEIC ACID) (10A) (IMMOBILIZ? OR ATTACH?) (10A) (DI VALENT OR MAGNESIUM)

=> s l1 and (chelat? or EDTA)

L2 0 L1 AND (CHELAT? OR EDTA)

=> dup rem l1

PROCESSING COMPLETED FOR L1

L3 15 DUP REM L1 (6 DUPLICATES REMOVED)

=> d l3 1-15 bib ab

L3 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2002 ACS

AN 2002:290241 CAPLUS

DN 137:2082

TI Mg2+-dependent conformational change of RNA studied by fluorescence correlation and FRET on immobilized single molecules

AU Kim, Harold D.; Nienhaus, G. Ulrich; Ha, Taekjip; Orr, Jeffrey W.; Williamson, James R.; Chu, Steven

CS Department of Applied Physics and Physics, Stanford University, Stanford, CA, 94305-4060, USA

SO Proceedings of the National Academy of Sciences of the United States of America (2002), 99(7), 4284-4289

CODEN: PNASA6; ISSN: 0027-8424

PB National Academy of Sciences

DT Journal

LA English

AB Fluorescence correlation spectroscopy (FCS) of fluorescence resonant energy transfer (FRET) on immobilized individual fluorophores was used to study the Mg2+-facilitated conformational change of an RNA three-helix junction, a structural element that initiates the folding of the 30S ribosomal subunit. Transitions of the RNA junction between open and folded conformations resulted in fluctuations in fluorescence by FRET. Fluorescence fluctuations occurring between two FRET states on the millisecond time scale were found to be dependent on Mg2+ and Na+ concns. Correlation functions of the fluctuations were used to det. transition rates between the two conformations as a function of Mg2+ or Na+ concn. Both the opening and folding rates were found to vary with changing salt

conditions. Assuming specific binding of divalent ions to RNA, the Mg²⁺ dependence of the obsd. rates cannot be explained by conformational change induced by Mg²⁺ binding/unbinding, but is consistent with a model in which the intrinsic conformational change of the RNA junction is altered by uptake of Mg²⁺ ion(s). This version of FCS/FRET on immobilized single mols. is demonstrated to be a powerful technique in the study of conformational dynamics of biomols. over time scales ranging from microseconds to seconds.

RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2002 ACS
AN 2001:526225 CAPLUS
DN 135:133079
TI Immobilization of oligonucleotides on nanoparticles and their use in nucleic acid hybridization
IN Mirkin, Chad A.; Letsinger, Robert L.; Mucic, Robert C.; Storhoff, James J.; Elghanian, Robert; Taton, Thomas Andrew; Li, Zhi
PA Nanosphere Inc., USA
SO PCT Int. Appl., 323 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 7

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------|---|------|----------|-----------------|----------|
| PI | WO 2001051665 | A2 | 20010719 | WO 2001-US1190 | 20010112 |
| | W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| | RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| PRAI | US 2000-176409P | P | 20000113 | | |
| | US 2000-200161P | P | 20000426 | | |
| | US 2000-603830 | A | 20000626 | | |
| | US 2001-760500 | A | 20010112 | | |
| AB | The invention provides methods of detecting a nucleic acid. The methods comprise contacting the nucleic acid with one or more types of particles having oligonucleotides attached thereto. In one embodiment of the method, the oligonucleotides are attached to nanoparticles and have sequences complementary to portions of the sequence of the nucleic acid. A detectable change (preferably a color change) is brought about as a result of the hybridization of the oligonucleotides on the nanoparticles to the nucleic acid. The invention also provides compns. and kits comprising particles. The invention further provides methods of synthesizing unique nanoparticle-oligonucleotide conjugates, the conjugates produced by the methods, and methods of using the conjugates. In addn., the invention provides nanomaterials and nanostructures comprising nanoparticles and methods of nanofabrication utilizing nanoparticles. Finally, the invention provides a method of sepg. a selected nucleic acid from other nucleic acids. The prepn. of colloidal gold nanoparticles with a diam. of 23 nm from HAuCl ₄ is described. Particles of this size show a color change upon aggregation. 3'-Thiol terminated oligonucleotides were immobilized on the surface of these particles. Oligonucleotide dependent aggregation and color changes were demonstrated and the hybridization conditions optimized. The prepn. of probe labeled quantum dots is also described. | | | | |

L3 ANSWER 3 OF 15 MEDLINE
AN 2001673528 MEDLINE

DUPLICATE 1

DN 21576336 PubMed ID: 11718557
TI Dynamic interactions of p53 with DNA in solution by time-lapse atomic force microscopy.
AU Jiao Y; Cherny D I; Heim G; Jovin T M; Schaffer T E
CS Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Gottingen, Germany.
SO JOURNAL OF MOLECULAR BIOLOGY, (2001 Nov 23) 314 (2) 233-43.
Journal code: 2985088R. ISSN: 0022-2836.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200112
ED Entered STN: 20011126
Last Updated on STN: 20020123
Entered Medline: 20011227
AB Dynamic interactions of the tumor suppressor protein p53 with a DNA fragment containing a p53-specific recognition sequence were directly observed by time-lapse tapping mode atomic force microscopy (AFM) in liquid. The **divalent cation Mg(2+)** was used to loosely attach both DNA and p53 to a mica surface so they could be imaged by the AFM while interacting with each other. Various interactions of p53 with DNA were observed, including dissociation/re-association, sliding and possibly direct binding to the specific sequence. Two modes of target recognition of p53 were detected: (a) direct binding, and (b) initial non-specific binding with subsequent translocation by one-dimensional diffusion of the protein along the DNA to the specific site.
Copyright 2001 Academic Press.

L3 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2002 ACS
AN 2000:401981 CAPLUS
DN 133:39077
TI Method for efficiently immobilizing DNA on a carrier using new buffer compositions
IN Takayama, Masanori; Rokushima, Masatomo; Ueda, Minoru; Okamoto, Sachiko; Ozaki, Aya; Mineno, Junichi; Kimizuka, Fusao; Asada, Kiyozo; Kato, Ikunoshin
PA Takara Shuzo Co., Ltd., Japan
SO PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DT Patent
LA Japanese
FAN.CNT 2

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------|---|--|----------|-----------------|----------|
| PI | WO 2000034456 | A1 | 20000615 | WO 1999-JP6865 | 19991208 |
| | W: | AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| | RW: | GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | |
| | EP 1138761 | A1 | 20011004 | EP 1999-959690 | 19991208 |
| | R: | AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | |
| PRAI | JP 1998-351276 | A | 19981210 | | |
| | WO 1999-JP6865 | W | 19991208 | | |
| AB | A method is described for efficiently immobilizing DNA (e.g., oligonucleotide, polynucleotide) on a carrier (e.g., glass, quartz) by having a step of bringing the DNA into contact with the carrier in a | | | | |

buffer contg. one or more substances selected from a group consisting of morpholine, morpholine deriv. (e.g., N-alkyl morpholine), their salts and a carbonate (e.g., sodium carbonate, potassium carbonate, magnesium carbonate, ammonium carbonate, triethylammonium carbonate). An addn. of a surfactant (e.g., nonionic surfactant, anionic surfactant, zwitterionic surfactant) to the buffer improves an efficiency of DNA immobilization. A larger amt. of DNA can be immobilized on the carrier by this method with improved immobilization rate and immobilized DNA d. comparing to the conventional methods. An improved sensitivity is achieved in detecting a target DNA by hybridization using the immobilized DNA prep. by this method.

RE.CNT 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 5 OF 15 MEDLINE DUPLICATE 2
AN 2000296792 MEDLINE
DN 20296792 PubMed ID: 10835269
TI Scanning force microscopy of the complexes of p53 core domain with supercoiled DNA.
AU Jett S D; Cherny D I; Subramaniam V; Jovin T M
CS Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, Gottingen, D-37077, Germany.
SO JOURNAL OF MOLECULAR BIOLOGY, (2000 Jun 9) 299 (3) 585-92.
Journal code: 2985088R. ISSN: 0022-2836.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200007
ED Entered STN: 20000720
Last Updated on STN: 20000720
Entered Medline: 20000711
AB We used scanning force microscopy to analyse the interaction of the core domain of the tumor suppressor protein p53 (p53CD, amino acid residues 94 to 312), with supercoiled DNA (scDNA) molecules. The complexes were attached to a mica substrate by the divalent cation spreading technique. p53CD bound to supercoiled plasmid pPGM1 bearing the consensus sequence 5'-AGACATGCCTAGACATGCCT-3' (p53CON) was imaged as a globular complex. Only one such complex was observed with each scDNA molecule. In contrast, binding to supercoiled pBluescript II SK(-) DNA (lacking the consensus sequence) resulted in the appearance of multiple, variable size complexes of various sizes on single DNA molecules. Addition of p53CD to scDNA containing a cruciform-forming (AT) (34) insert resulted in the binding of the protein exclusively at the cruciform. The data presented here suggest that p53CD can form stable specific and non-specific complexes with supercoiled DNA molecules, albeit of variable multimeric organization.
Copyright 2000 Academic Press.

L3 ANSWER 6 OF 15 MEDLINE DUPLICATE 3
AN 2000042578 MEDLINE
DN 20042578 PubMed ID: 10571996
TI RNA tertiary folding monitored by fluorescence of covalently attached pyrene.
AU Silverman S K; Cech T R
CS Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Colorado at Boulder 80309-0215, USA..
Scott.Silverman@colorado.edu
NC GM28039 (NIGMS)
SO BIOCHEMISTRY, (1999 Oct 26) 38 (43) 14224-37.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English

FS Priority Journals
 EM 199912
 ED Entered STN: 20000113
 Last Updated on STN: 20000113
 Entered Medline: 19991217
 AB The pathways by which large RNAs adopt tertiary structure are just beginning to be explored, and new methods that reveal RNA folding are highly desirable. Here we report an assay for RNA tertiary folding in which the fluorescence of a covalently incorporated chromophore is monitored. Folding of the 160-nucleotide Tetrahymena group I intron P4-P6 domain was used as a test system. Guided by the P4-P6 X-ray crystal structure, we chose a nucleotide (U107) for which derivatization at the 2'-position should not perturb the folded conformation. A 15-mer RNA oligonucleotide with a 2'-amino substitution at U107 was derivatized with a pyrene chromophore on a variable-length tether, and then ligated to the remainder of P4-P6, providing a site-specifically pyrene-labeled P4-P6 derivative. Upon titration of the pyrene-derivatized P4-P6 with Mg(2+), the equilibrium fluorescence intensity reversibly increased several-fold, as expected if the probe's chemical microenvironment changes as the RNA to which it is attached folds. The concentration and specificity of divalent ions required to induce the fluorescence change (Mg(2+) approximately Ca(2+) > Sr(2+)) correlated well with biochemical folding assays that involve nondenaturing gel electrophoresis. Furthermore, mutations in P4-P6 remote from the chromophore that shifted the Mg(2+) folding requirement on nondenaturing gels also affected in a predictable way the Mg(2+) requirement for the fluorescence increase. Initial stopped-flow studies with millisecond time resolution suggest that this fluorescence method will be useful for following the kinetics of P4-P6 tertiary folding. We conclude that a single site-specifically tethered chromophore can report the formation of global structure of a large RNA molecule, allowing one to monitor both the equilibrium progress and the real-time kinetics of RNA tertiary folding.

L3 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2002 ACS
 AN 1997:296934 CAPLUS
 DN 126:273255
 TI Integrase ribonucleoprotein particles comprising group II intron RNA and encoded protein for DNA cleavage and attachment of nucleic acid to cleaved DNA
 IN Lambowitz, Alan M.; Zimmerly, Steven; Yang, Jian; Guo, Huatao
 PA Ohio State Research Foundation, USA
 SO PCT Int. Appl., 57 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 3

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|----|---|------|----------|-----------------|----------|
| PI | WO 9710362 | A1 | 19970320 | WO 1996-US14609 | 19960911 |
| | W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| | RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR | | | | |
| US | 5698421 | A | 19971216 | US 1995-526964 | 19950912 |
| AU | 9669744 | A1 | 19970401 | AU 1996-69744 | 19960911 |
| AU | 715563 | B2 | 20000203 | | |
| EP | 851940 | A1 | 19980708 | EP 1996-930830 | 19960911 |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| JP | 11512291 | T2 | 19991026 | JP 1996-512092 | 19960911 |
| US | 5869634 | A | 19990209 | US 1997-946617 | 19971007 |

PRAI US 1995-526964 A 19950912
WO 1996-US14609 W 19960911

AB The present invention provides new methods, employing a nucleotide integrase, for cleaving double-stranded and single-stranded DNA substrates at specific sites and for attaching nucleic acid mols. to the cleaved DNA substrates. One method uses a nucleotide integrase to cleave one strand of a double-stranded DNA and to concomitantly attach a nucleic acid mol. to the cleaved strand. Another method uses a nucleotide integrase to cleave both strands of a double-stranded DNA substrate and to attach a nucleic acid mol. to one strand of the DNA substrate. Another method uses a nucleotide integrase to cleave both strands of a double-stranded DNA substrate and to attach an RNA mol. to one strand of the substrate and for attaching a cDNA to the other strand of the substrate. Another method cleaves single stranded DNA with the concomitant insertion of a nucleic acid mol. at the cleavage point. The nucleotide integrase comprises an RNP particle which comprises a group II intron RNA bound to a group II intron encoded protein. The present invention also relates to purified and reconstituted RNP particles and reconstituted RNP that cleave DNA substrates.

L3 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2002 ACS
AN 1994:572091 CAPLUS

DN 121:172091

TI Ordered assembly of a functional preinitiation transcription complex, containing vaccinia virus early transcription factor and RNA polymerase, on an immobilized template

AU Baldick, Carl J., Jr.; Cassetti, Maria Cristina; Harris, Nicholas; Moss, Bernard

CS Lab. Viral Diseases, Natl. Inst. Allergy Infect. Diseases, Bethesda, MD, 20892, USA

SO Journal of Virology (1994), 68(9), 6052-6
CODEN: JOVIAM; ISSN: 0022-538X

DT Journal

LA English

AB A functional preinitiation transcription complex was formed by incubating vaccinia virus early transcription factor VETF and RAP94+ RNA polymerase with an early promoter template immobilized on paramagnetic particles. A preferred order of assembly, VETF followed by RNA polymerase, was demonstrated by stepwise addn. expts. ATP was unnecessary for preinitiation transcription complex formation, but divalent cations were required specifically for the assocn. of RNA polymerase.

L3 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2002 ACS
AN 1994:650332 CAPLUS

DN 121:250332

TI Mg²⁺-selective electrode comprising double-helical DNA as receptive entity

AU Maeda, Mizuo; Nakano, Koji; Uchida, Shinji; Takagi, Makoto

CS Faculty of Engineering, Kyushu University, Fukuoka, 812, Japan

SO Chemistry Letters (1994), (10), 1805-8

CODEN: CMLTAG; ISSN: 0366-7022

DT Journal

LA English

AB Cyclic voltammograms of ferrocyanide/ferricyanide redox couple with a DNA-immobilized electrode gave the peak currents due to the reversible electrode reaction, which were significantly enhanced on adding Mg²⁺. The electrode responded also to Ca²⁺ and Ba²⁺, although the onset concns. of the electrode response were 50-times larger than that for Mg²⁺. The selectivity in the order of Mg²⁺ > Ca²⁺, Ba²⁺ >> Na⁺, K⁺ seems consistent with the binding affinity of the metal ions with double-helical DNA.

L3 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2002 ACS
AN 1991:225170 CAPLUS

DN 114:225170

TI Metal oxide supports for nucleic acids

IN Bitner, Rex M. C.; Funkenbusch, Eric F.
PA Minnesota Mining and Mfg. Co., USA
SO Eur. Pat. Appl., 15 pp.
CODEN: EPXXDW

DT Patent
LA English

FAN.CNT 1

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------|---------------------------------------|------|----------|-----------------|----------|
| PI | EP 391608 | A2 | 19901010 | EP 1990-303382 | 19900329 |
| | EP 391608 | A3 | 19920415 | | |
| | R: BE, CH, DE, FR, GB, IT, LI, NL, SE | | | | |
| | CA 2011929 | AA | 19901003 | CA 1990-2011929 | 19900312 |
| | AU 9051268 | A1 | 19901004 | AU 1990-51268 | 19900312 |
| | JP 02286100 | A2 | 19901126 | JP 1990-88107 | 19900402 |
| PRAI | US 1989-332541 | | 19890403 | | |

AB Nucleic acids are sorbed to metal oxide supports with sufficient strength and in sufficient amts. to enable the resulting compns. of matter to be used for such purposes as hybridizing, labeling, sequencing, and synthesis of nucleic acids. The compns. can optionally also be bound with blocking agent to prevent undesired nucleic acid sorption, e.g., of probe nucleic acids in a hybridization expt. Preferred compns. are easily prep'd. and used, versatile, and reusable. Sorption of .lambda. phage DNA to, e.g., ZrO₂ for a hybridization assay is described, as is prepn. of a ZrO₂-PTFE composite structure and its use in a Southern blot hybridization procedure.

L3 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2002 ACS

AN 1990:95110 CAPLUS

DN 112:95110

TI Method and kit for reversibly staining immobilized and enzymically-labeled nucleic acids using sulfur-containing substrates and metals

IN Lebacq, Philippe
PA Bioprobe Systems, Fr.
SO Eur. Pat. Appl., 8 pp.
CODEN: EPXXDW

DT Patent
LA French

FAN.CNT 1

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------|---|------|----------|-----------------|----------|
| PI | EP 334756 | A1 | 19890927 | EP 1989-400801 | 19890321 |
| | EP 334756 | B1 | 19920624 | | |
| | R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| | FR 2629100 | A1 | 19890929 | FR 1988-3982 | 19880325 |
| | FR 2629100 | B1 | 19930820 | | |
| | FR 2680374 | A1 | 19930219 | FR 1989-3191 | 19890310 |
| | FR 2680374 | B1 | 19931112 | | |
| | AT 77654 | E | 19920715 | AT 1989-400801 | 19890321 |
| | US 5073483 | A | 19911217 | US 1989-328340 | 19890324 |
| | JP 02009400 | A2 | 19900112 | JP 1989-72017 | 19890327 |
| PRAI | FR 1988-3982 | | 19880325 | | |
| | FR 1989-3191 | | 19890310 | | |
| | EP 1989-400801 | | 19890321 | | |

AB A method and kit for reversibly staining a nucleic acid sequence immobilized on a solid support comprises: (1) using an enzyme system (e.g. contg. alk. phosphatase) which is (in)directly bonded to the nucleic acid sequence as nonradioactive label; (2) reacting the enzyme with a S-contg. org. substrate to form a thiol group-contg. product; and (3) reacting the product with a metal (e.g. Au) compd. which is sol. and stable in an aq. soln. to form a pptd. metal-S-contg. org. compd. complex at the site of the nucleic acid sequence. The complex can be further reacted with a compd. to accentuate the coloration and/or with a decoloration soln. contg. Na₂S₂O₃ and (NH₄)₂S₂O₃. A membrane-immobilized and alk.

phosphatase-labeled nucleic acid was reacted with color developing soln. contg. pH 9.5 Tris-acetate 50, Mg acetate 10, cysteamine phosphate 5, and aurothioglucose 3 mM for 15 min - 2 h. HClO₄ or AgNO₃ was used to enhance the visualization, by changing the color from lemon yellow/gold yellow to chestnut brown. By this method, it is possible to detect 1 pg DNA in dot blot hybridization and visualize single genomic DNA sequence after Southern transfer.

L3 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2002 ACS
AN 1984:546793 CAPLUS
DN 101:146793
TI Poly(ADP-ribose) synthetase
AU Ueda, Kunihiro; Zhang, Jingyuan; Hayaishi, Osamu
CS Fac. Med., Kyoto Univ., Kyoto, 606, Japan
SO Methods Enzymol. (1984), 106(Posttransl. Modif., Part A), 500-4
CODEN: MENZAU; ISSN: 0076-6879
DT Journal
LA English
AB A method for the prepn. of immobilized poly(ADP-ribose) synthetase is presented. DNA is required for the immobilized enzyme activity. Histone H1 inhibited the automodification of the immobilized enzyme in the presence of Mg²⁺, and slightly stimulated it in the absence of Mg²⁺. The product polymer synthesized on the immobilized enzyme had branches.

L3 ANSWER 13 OF 15 MEDLINE DUPLICATE 4
AN 77045491 MEDLINE
DN 77045491 PubMed ID: 990263
TI Poly(adenylic acid) synthesis in isolated rat liver mitochondria.
AU Rose K M; Jacob S T
SO BIOCHEMISTRY, (1976 Nov 16) 15 (23) 5046-52.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 197701
ED Entered STN: 19900313
Last Updated on STN: 19970203
Entered Medline: 19770129
AB Purified rat liver mitochondria were shown to synthesize poly(adenylic acid) (poly(A)) in vitro. Detection of the poly(A) synthesizing activity was facilitated by addition of NaF to the reaction was shown to be poly(A) by its insensitivity to digestion with pancreatic RNase and RNase T1, its degradation by venom phosphodiesterase and its retention on poly (uridylic acid) 20-23 AMP units and it was covalently attached to the endogenous RNA in the mitochondria. Poly(A) synthesis required ATP and a divalent ion and was maximally active in the pH range of 7-8. The reaction was inhibited by atractyloside, cordycepin triphosphate, Rose Bengal, rifamycin derivative AF/103, sodium pyrophosphate, and N-ethylmaleimide. These studies indicate that the mitochondrial poly(A) polymerase previously described in our laboratory (Jacob, S.T., Rose, K.M., and Morris, H.P. (1974), Biochim. Biophys. Acta 361, 312-320) is involved in the posttranscriptional addition of poly(A) sequence to mitochondrial RNA.

L3 ANSWER 14 OF 15 MEDLINE
AN 76053079 MEDLINE
DN 76053079 PubMed ID: 1059099
TI Chromatin and DNA synthesis associated with nuclear membrane in germinating cotton.
AU Clay W F; Katterman F R; Bartels P G
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1975 Aug) 72 (8) 3134-8.
Journal code: 7505876. ISSN: 0027-8424.

CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 197601
ED Entered STN: 19900313
Last Updated on STN: 19900313
Entered Medline: 19760116
AB The synthesis of nuclear DNA and possible attachment sites of chromatin in the cells of cotton (*Gossypium barbadense*) radicles during germination was investigated. Biochemical analysis of nuclear membrane fragments or Sarkosyl-magnesium-membrane complexes indicates that the DNA, including newly replicated DNA, is attached to the nuclear membranes during periods of active synthesis. Electron micrographs of nuclear membrane fragments indicate a physical association between chromatin fibers and the membranes. The attachment site appears to be proteinaceous; since the chromatin is released by protein degradative enzymes as evidenced by biochemical techniques and electron microscopic observations. Short-term labeling results in incorporation into a membrane-associated product indistinguishable from the bulk of nuclear DNA. DNA polymerase activity is also associated with nuclear membrane preparations in which [³H]thymidine triphosphate is incorporated into an acid-insoluble. DNase-sensitive product.

L3 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1974:95573 BIOSIS
DN BR10:95573
TI STRUCTURAL STUDIES OF BDELLOVIBRIO-BACTERIOVORUS STRAIN 109 HOST INTERACTION WHEN PENETRATION IS PREVENTED.
AU ABRAM D; CASTRO E MELO J
SO Abstr. Annu. Meet. Am. Soc. Microbiol., (1974) 74, 63.
CODEN: ASMACK. ISSN: 0094-8519.
DT Conference
FS BR; OLD
LA Unavailable

=> d 13 1-15 bib ab kwic

L3 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2002 ACS
AN 2002:290241 CAPLUS
DN 137:2082
TI Mg²⁺-dependent conformational change of RNA studied by fluorescence correlation and FRET on immobilized single molecules
AU Kim, Harold D.; Nienhaus, G. Ulrich; Ha, Taekjip; Orr, Jeffrey W.; Williamson, James R.; Chu, Steven
CS Department of Applied Physics and Physics, Stanford University, Stanford, CA, 94305-4060, USA
SO Proceedings of the National Academy of Sciences of the United States of America (2002), 99(7), 4284-4289
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
AB Fluorescence correlation spectroscopy (FCS) of fluorescence resonant energy transfer (FRET) on immobilized individual fluorophores was used to study the Mg²⁺-facilitated conformational change of an RNA three-helix junction, a structural element that initiates the folding of the 30S ribosomal subunit. Transitions of the RNA junction between open and folded conformations resulted in fluctuations in fluorescence by FRET. Fluorescence fluctuations occurring between two FRET states on the millisecond time scale were found to be dependent on Mg²⁺ and Na⁺ concns. Correlation functions of the fluctuations were used to det. transition rates between the two conformations as a function of Mg²⁺ or Na⁺ concn.

Both the opening and folding rates were found to vary with changing salt conditions. Assuming specific binding of divalent ions to RNA, the Mg²⁺ dependence of the obsd. rates cannot be explained by conformational change induced by Mg²⁺ binding/unbinding, but is consistent with a model in which the intrinsic conformational change of the RNA junction is altered by uptake of Mg²⁺ ion(s). This version of FCS/FRET on immobilized single mols. is demonstrated to be a powerful technique in the study of conformational dynamics of biomols. over time scales ranging from microseconds to seconds.

RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

IT Conformational transition

(magnesium and sodium ions play role in conformational change of RNA studied by fluorescence correlation and FRET on immobilized single mols.)

IT RNA

RL: BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); PYP (Physical process); BIOL (Biological study); PROC (Process)

(magnesium and sodium ions play role in conformational change of RNA studied by fluorescence correlation and FRET on immobilized single mols.)

IT 7439-95-4, Magnesium, biological studies 7440-23-5, Sodium, biological studies

RL: BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); PYP (Physical process); BIOL (Biological study); PROC (Process)

(magnesium and sodium ions play role in conformational change of RNA studied by fluorescence correlation and FRET on immobilized single mols.)

L3 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2002 ACS

AN 2001:526225 CAPLUS

DN 135:133079

TI Immobilization of oligonucleotides on nanoparticles and their use in nucleic acid hybridization

IN Mirkin, Chad A.; Letsinger, Robert L.; Mucic, Robert C.; Storhoff, James J.; Elghanian, Robert; Taton, Thomas Andrew; Li, Zhi

PA Nanosphere Inc., USA

SO PCT Int. Appl., 323 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 7

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------|-----------------|--|----------|-----------------|----------|
| PI | WO 2001051665 | A2 | 20010719 | WO 2001-US1190 | 20010112 |
| | W: | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| | RW: | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | |
| PRAI | US 2000-176409P | P | 20000113 | | |
| | US 2000-200161P | P | 20000426 | | |
| | US 2000-603830 | A | 20000626 | | |
| | US 2001-760500 | A | 20010112 | | |

AB The invention provides methods of detecting a nucleic acid. The methods comprise contacting the nucleic acid with one or more types of particles having oligonucleotides attached thereto. In one embodiment of the method, the oligonucleotides are attached to nanoparticles and have

sequences complementary to portions of the sequence of the nucleic acid. A detectable change (preferably a color change) is brought about as a result of the hybridization of the oligonucleotides on the nanoparticles to the nucleic acid. The invention also provides compns. and kits comprising particles. The invention further provides methods of synthesizing unique nanoparticle-oligonucleotide conjugates, the conjugates produced by the methods, and methods of using the conjugates. In addn., the invention provides nanomaterials and nanostructures comprising nanoparticles and methods of nanofabrication utilizing nanoparticles. Finally, the invention provides a method of sepg. a selected nucleic acid from other nucleic acids. The prepn. of colloidal gold nanoparticles with a diam. of 23 nm from HAuCl₄ is described. Particles of this size show a color change upon aggregation. 3'-Thiol terminated oligonucleotides were immobilized on the surface of these particles. Oligonucleotide dependent aggregation and color changes were demonstrated and the hybridization conditions optimized. The prepn. of probe labeled quantum dots is also described.

IT 127-09-3, Sodium acetate 631-61-8, Ammonium acetate 7447-40-7, Potassium chloride, uses 7647-14-5, Sodium chloride, uses 7786-30-3, Magnesium chloride, uses 12125-02-9, Ammonium chloride, uses RL: MOA (Modifier or additive use); USES (Uses)
(in oligonucleotide binding to gold nanoparticles;
immobilization of oligonucleotides on nanoparticles and their
use in nucleic acid hybridization)

L3 ANSWER 3 OF 15 MEDLINE DUPLICATE 1
AN 2001673528 MEDLINE
DN 21576336 PubMed ID: 11718557
TI Dynamic interactions of p53 with DNA in solution by time-lapse atomic force microscopy.
AU Jiao Y; Cherny D I; Heim G; Jovin T M; Schaffter T E
CS Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Gottingen, Germany.
SO JOURNAL OF MOLECULAR BIOLOGY, (2001 Nov 23) 314 (2) 233-43.
Journal code: 2985088R. ISSN: 0022-2836.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200112
ED Entered STN: 20011126
Last Updated on STN: 20020123
Entered Medline: 20011227
AB Dynamic interactions of the tumor suppressor protein p53 with a DNA fragment containing a p53-specific recognition sequence were directly observed by time-lapse tapping mode atomic force microscopy (AFM) in liquid. The **divalent cation Mg(2+)** was used to loosely attach both **DNA** and p53 to a mica surface so they could be imaged by the AFM while interacting with each other. Various interactions of p53 with DNA were observed, including dissociation/re-association, sliding and possibly direct binding to the specific sequence. Two modes of target recognition of p53 were detected: (a) direct binding, and (b) initial non-specific binding with subsequent translocation by one-dimensional diffusion of the protein along the DNA to the specific site.
Copyright 2001 Academic Press.
AB . . . fragment containing a p53-specific recognition sequence were directly observed by time-lapse tapping mode atomic force microscopy (AFM) in liquid. The **divalent cation Mg(2+)** was used to loosely attach both **DNA** and p53 to a mica surface so they could be imaged by the AFM while interacting with each other. Various. . .

L3 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2002 ACS
AN 2000:401981 CAPLUS

DN 133:39077
TI Method for efficiently immobilizing DNA on a carrier using new buffer compositions
IN Takayama, Masanori; Rokushima, Masatomo; Ueda, Minoru; Okamoto, Sachiko; Ozaki, Aya; Mineno, Junichi; Kimizuka, Fusao; Asada, Kiyozo; Kato, Ikuunoshin
PA Takara Shuzo Co., Ltd., Japan
SO PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DT Patent
LA Japanese
FAN.CNT 2

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|----|--|------|----------|-----------------|----------|
| PI | WO 2000034456 | A1 | 20000615 | WO 1999-JP6865 | 19991208 |
| | W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| | EP 1138761 | A1 | 20011004 | EP 1999-959690 | 19991208 |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | | |

PRAI JP 1998-351276 A 19981210
WO 1999-JP6865 W 19991208

AB A method is described for efficiently immobilizing DNA (e.g., oligonucleotide, polynucleotide) on a carrier (e.g., glass, quartz) by having a step of bringing the DNA into contact with the carrier in a buffer contg. one or more substances selected from a group consisting of morpholine, morpholine deriv. (e.g., N-alkyl morpholine), their salts and a carbonate (e.g., sodium carbonate, potassium carbonate, magnesium carbonate, ammonium carbonate, triethylammonium carbonate). An addn. of a surfactant (e.g., nonionic surfactant, anionic surfactant, zwitterionic surfactant) to the buffer improves an efficiency of DNA immobilization. A larger amt. of DNA can be immobilized on the carrier by this method with improved immobilization rate and immobilized DNA d. comparing to the conventional methods. An improved sensitivity is achieved in detecting a target DNA by hybridization using the immobilized DNA prep'd. by this method.

RE.CNT 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

IT 83-44-3 107-97-1, Sarcosine 109-02-4, N-Methyl morpholine 110-91-8, Morpholine, uses 110-91-8D, Morpholine, deriv.; n-alkyl, uses 151-21-3, Sodium dodecylsulfate, uses 361-09-1, Sodium cholate 497-19-8, Sodium carbonate, uses 506-87-6, Ammonium carbonate 546-93-0, Magnesium carbonate 584-08-7, Potassium carbonate 3812-32-6, Carbonate, uses 9002-93-1, Triton X-100 9036-19-5, Nonidet P-40 11024-24-1, Digitonin 14808-60-7, Quartz (SiO₂), uses 15715-57-8, Triethylammonium carbonate 25339-99-5, Sucrose monolaurate 31835-06-0, Sucrose monocaprate 69227-93-6, n-Dodecyl-.beta.-D-maltoside 75621-03-3, CHAPS 82473-24-3, CHAPSO 85261-20-7, MEGA-10 85618-20-8 85618-21-9 86303-22-2, BIGCHAP 86303-23-3, Deoxy-BIGCHAP
RL: NUU (Other use, unclassified); USES (Uses)
(method for efficiently immobilizing DNA on carrier using new buffer compns.)

L3 ANSWER 5 OF 15 MEDLINE DUPLICATE 2
AN 2000296792 MEDLINE
DN 20296792 PubMed ID: 10835269
TI Scanning force microscopy of the complexes of p53 core domain with

supercoiled DNA.

AU Jett S D; Cherny D I; Subramaniam V; Jovin T M
CS Department of Molecular Biology, Max Planck Institute for Biophysical
Chemistry, Am Fassberg 11, Gottingen, D-37077, Germany.
SO JOURNAL OF MOLECULAR BIOLOGY, (2000 Jun 9) 299 (3) 585-92.
Journal code: 2985088R. ISSN: 0022-2836.

CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200007
ED Entered STN: 20000720
Last Updated on STN: 20000720
Entered Medline: 20000711

AB We used scanning force microscopy to analyse the interaction of the core domain of the tumor suppressor protein p53 (p53CD, amino acid residues 94 to 312), with supercoiled DNA (scDNA) molecules. The complexes were attached to a mica substrate by the **divalent** cation spreading technique. p53CD bound to supercoiled plasmid pPGM1 bearing the consensus sequence 5'-AGACATGCCTAGACATGCCT-3' (p53CON) was imaged as a globular complex. Only one such complex was observed with each scDNA molecule. In contrast, binding to supercoiled pBluescript II SK(-) DNA (lacking the consensus sequence) resulted in the appearance of multiple, variable size complexes of various sizes on single DNA molecules. Addition of p53CD to scDNA containing a cruciform-forming (AT) (34) insert resulted in the binding of the protein exclusively at the cruciform. The data presented here suggest that p53CD can form stable specific and non-specific complexes with supercoiled DNA molecules, albeit of variable multimeric organization.
Copyright 2000 Academic Press.

AB . . . interaction of the core domain of the tumor suppressor protein p53 (p53CD, amino acid residues 94 to 312), with supercoiled DNA (scDNA) molecules. The complexes were attached to a mica substrate by the **divalent** cation spreading technique. p53CD bound to supercoiled plasmid pPGM1 bearing the consensus sequence 5'-AGACATGCCTAGACATGCCT-3' (p53CON) was imaged as a globular. . .

L3 ANSWER 6 OF 15 MEDLINE DUPLICATE 3
AN 2000042578 MEDLINE
DN 20042578 PubMed ID: 10571996
TI RNA tertiary folding monitored by fluorescence of covalently attached pyrene.
AU Silverman S K; Cech T R
CS Howard Hughes Medical Institute, Department of Chemistry and Biochemistry,
University of Colorado at Boulder 80309-0215, USA..
Scott.Silverman@colorado.edu
NC GM28039 (NIGMS)
SO BIOCHEMISTRY, (1999 Oct 26) 38 (43) 14224-37.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199912
ED Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991217

AB The pathways by which large RNAs adopt tertiary structure are just beginning to be explored, and new methods that reveal RNA folding are highly desirable. Here we report an assay for RNA tertiary folding in which the fluorescence of a covalently incorporated chromophore is monitored. Folding of the 160-nucleotide Tetrahymena group I intron P4-P6 domain was used as a test system. Guided by the P4-P6 X-ray crystal structure, we chose a nucleotide (U107) for which derivatization at the

2'-position should not perturb the folded conformation. A 15-mer RNA oligonucleotide with a 2'-amino substitution at U107 was derivatized with a pyrene chromophore on a variable-length tether, and then ligated to the remainder of P4-P6, providing a site-specifically pyrene-labeled P4-P6 derivative. Upon titration of the pyrene-derivatized P4-P6 with Mg(2+), the equilibrium fluorescence intensity reversibly increased several-fold, as expected if the probe's chemical microenvironment changes as the RNA to which it is attached folds. The concentration and specificity of divalent ions required to induce the fluorescence change (Mg(2+) approximately Ca(2+) > Sr(2+)) correlated well with biochemical folding assays that involve nondenaturing gel electrophoresis. Furthermore, mutations in P4-P6 remote from the chromophore that shifted the Mg(2+) folding requirement on nondenaturing gels also affected in a predictable way the Mg(2+) requirement for the fluorescence increase. Initial stopped-flow studies with millisecond time resolution suggest that this fluorescence method will be useful for following the kinetics of P4-P6 tertiary folding. We conclude that a single site-specifically tethered chromophore can report the formation of global structure of a large RNA molecule, allowing one to monitor both the equilibrium progress and the real-time kinetics of RNA tertiary folding.

AB . . . P4-P6 with Mg(2+), the equilibrium fluorescence intensity reversibly increased several-fold, as expected if the probe's chemical microenvironment changes as the RNA to which it is attached folds. The concentration and specificity of divalent ions required to induce the fluorescence change (Mg(2+) approximately Ca(2+) > Sr(2+)) correlated well with biochemical folding assays that involve. . .

L3 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2002 ACS
 AN 1997:296934 CAPLUS
 DN 126:273255
 TI Integrase ribonucleoprotein particles comprising group II intron RNA and encoded protein for DNA cleavage and attachment of nucleic acid to cleaved DNA
 IN Lambowitz, Alan M.; Zimmerly, Steven; Yang, Jian; Guo, Huatao
 PA Ohio State Research Foundation, USA
 SO PCT Int. Appl., 57 pp.
 CODEN: PIXXD2
 DT Patent
 LA English

FAN.CNT 3

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------|---|------|----------|-----------------|----------|
| PI | WO 9710362 | A1 | 19970320 | WO 1996-US14609 | 19960911 |
| | W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| | RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR | | | | |
| US | 5698421 | A | 19971216 | US 1995-526964 | 19950912 |
| AU | 9669744 | A1 | 19970401 | AU 1996-69744 | 19960911 |
| AU | 715563 | B2 | 20000203 | | |
| EP | 851940 | A1 | 19980708 | EP 1996-930830 | 19960911 |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| JP | 11512291 | T2 | 19991026 | JP 1996-512092 | 19960911 |
| US | 5869634 | A | 19990209 | US 1997-946617 | 19971007 |
| PRAI | US 1995-526964 | A | 19950912 | | |
| | WO 1996-US14609 | W | 19960911 | | |

AB The present invention provides new methods, employing a nucleotide integrase, for cleaving double-stranded and single-stranded DNA substrates at specific sites and for attaching nucleic acid mols. to the cleaved DNA

substrates. One method uses a nucleotide integrase to cleave one strand of a double-stranded DNA and to concomitantly attach a nucleic acid mol. to the cleaved strand. Another method uses a nucleotide integrase to cleave both strands of a double-stranded DNA substrate and to attach a nucleic acid mol. to one strand of the DNA substrate. Another method uses a nucleotide integrase to cleave both strands of a double-stranded DNA substrate and to attach an RNA mol. to one strand of the substrate and for attaching a cDNA to the other strand of the substrate. Another method cleaves single stranded DNA with the concomitant insertion of a nucleic acid mol. at the cleavage point. The nucleotide integrase comprises an RNP particle which comprises a group II intron RNA bound to a group II intron encoded protein. The present invention also relates to purified and reconstituted RNP particles and reconstituted RNP that cleave DNA substrates.

IT Cations

(**divalent; integrase ribonucleoprotein particles comprising group II intron RNA and encoded protein for DNA cleavage and attachment of nucleic acid to cleaved DNA**)

L3 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2002 ACS

AN 1994:572091 CAPLUS

DN 121:172091

TI Ordered assembly of a functional preinitiation transcription complex, containing vaccinia virus early transcription factor and RNA polymerase, on an immobilized template

AU Baldick, Carl J., Jr.; Cassetti, Maria Cristina; Harris, Nicholas; Moss, Bernard

CS Lab. Viral Diseases, Natl. Inst. Allergy Infect. Diseases, Bethesda, MD, 20892, USA

SO Journal of Virology (1994), 68(9), 6052-6

CODEN: JOVIAM; ISSN: 0022-538X

DT Journal

LA English

AB A functional preinitiation transcription complex was formed by incubating vaccinia virus early transcription factor VETF and RAP94+ RNA polymerase with an early promoter template immobilized on paramagnetic particles. A preferred order of assembly, VETF followed by RNA polymerase, was demonstrated by stepwise addn. expts. ATP was unnecessary for preinitiation transcription complex formation, but divalent cations were required specifically for the assocn. of RNA polymerase.

IT Transcription, genetic

(ordered assembly of functional preinitiation complex of, **immobilized template contg. vaccinia virus early transcription factor and RNA polymerase in relation to, divalent cation requirement in relation to**)

IT Virus, animal

(vaccinia, early transcription factor and RNA polymerase of, ordered assembly of functional preinitiation complex of, **immobilized template in relation to, divalent cation requirement in relation to**)

L3 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2002 ACS

AN 1994:650332 CAPLUS

DN 121:250332

TI Mg²⁺-selective electrode comprising double-helical DNA as receptive entity

AU Maeda, Mizuo; Nakano, Koji; Uchida, Shinji; Takagi, Makoto

CS Faculty of Engineering, Kyushu University, Fukuoka, 812, Japan

SO Chemistry Letters (1994), (10), 1805-8

CODEN: CMLTAG; ISSN: 0366-7022

DT Journal

LA English

AB Cyclic voltammograms of ferrocyanide/ferricyanide redox couple with a DNA-immobilized electrode gave the peak currents due to the reversible electrode reaction, which were significantly enhanced on adding Mg²⁺. The

electrode responded also to Ca²⁺ and Ba²⁺, although the onset concns. of the electrode response were 50-times larger than that for Mg²⁺. The selectivity in the order of Mg²⁺ > Ca²⁺, Ba²⁺ >> Na⁺, K⁺ seems consistent with the binding affinity of the metal ions with double-helical DNA.

ST DNA immobilization electrode magnesium

L3 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2002 ACS

AN 1991:225170 CAPLUS

DN 114:225170

TI Metal oxide supports for nucleic acids

IN Bitner, Rex M. C.; Funkenbusch, Eric F.

PA Minnesota Mining and Mfg. Co., USA

SO Eur. Pat. Appl., 15 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|----|---------------------------------------|------|----------|-----------------|----------|
| PI | EP 391608 | A2 | 19901010 | EP 1990-303382 | 19900329 |
| | EP 391608 | A3 | 19920415 | | |
| | R: BE, CH, DE, FR, GB, IT, LI, NL, SE | | | | |
| | CA 2011929 | AA | 19901003 | CA 1990-2011929 | 19900312 |
| | AU 9051268 | A1 | 19901004 | AU 1990-51268 | 19900312 |
| | JP 02286100 | A2 | 19901126 | JP 1990-88107 | 19900402 |

PRAI US 1989-332541 19890403

AB Nucleic acids are sorbed to metal oxide supports with sufficient strength and in sufficient amts. to enable the resulting compns. of matter to be used for such purposes as hybridizing, labeling, sequencing, and synthesis of nucleic acids. The compns. can optionally also be bound with blocking agent to prevent undesired nucleic acid sorption, e.g., of probe nucleic acids in a hybridization expt. Preferred compns. are easily prep'd. and used, versatile, and reusable. Sorption of .lambda. phage DNA to, e.g., ZrO₂ for a hybridization assay is described, as is prepn. of a ZrO₂-PTFE composite structure and its use in a Southern blot hybridization procedure.

IT 37230-85-6, Hafnium oxide 1305-78-8, Calcium oxide, biological studies 1307-96-6, Cobalt oxide, biological studies 1309-48-4, Magnesium oxide, biological studies 1312-81-8, Lanthanum oxide 1313-13-9, Manganese oxide, biological studies 1313-99-1, Nickel oxide, biological studies 1314-13-2, Zinc oxide, biological studies 1314-23-4, Zirconium oxide, biological studies 1314-36-9, Yttrium oxide, biological studies 1332-37-2, Iron oxide, biological studies 1344-28-1, Aluminum oxide, biological studies 13463-67-7, Titanium oxide, biological studies

RL: ANST (Analytical study)

(nucleic acid immobilization on support
of)

L3 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2002 ACS

AN 1990:95110 CAPLUS

DN 112:95110

TI Method and kit for reversibly staining immobilized and enzymically-labeled nucleic acids using sulfur-containing substrates and metals

IN Lebacq, Philippe

PA Bioprobe Systems, Fr.

SO Eur. Pat. Appl., 8 pp.

CODEN: EPXXDW

DT Patent

LA French

FAN.CNT 1

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|----|------------|------|----------|-----------------|----------|
| PI | EP 334756 | A1 | 19890927 | EP 1989-400801 | 19890321 |
| | EP 334756 | B1 | 19920624 | | |

R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE
 FR 2629100 A1 19890929 FR 1988-3982 19880325
 FR 2629100 B1 19930820
 FR 2680374 A1 19930219 FR 1989-3191 19890310
 FR 2680374 B1 19931112
 AT 77654 E 19920715 AT 1989-400801 19890321
 US 5073483 A 19911217 US 1989-328340 19890324
 JP 02009400 A2 19900112 JP 1989-72017 19890327
 PRAI FR 1988-3982 19880325
 FR 1989-3191 19890310
 EP 1989-400801 19890321
 AB A method and kit for reversibly staining a nucleic acid sequence immobilized on a solid support comprises: (1) using an enzyme system (e.g. contg. alk. phosphatase) which is (in)directly bonded to the nucleic acid sequence as nonradioactive label; (2) reacting the enzyme with a S-contg. org. substrate to form a thiol group-contg. product; and (3) reacting the product with a metal (e.g. Au) compd. which is sol. and stable in an aq. soln. to form a pptd. metal-S-contg. org. compd. complex at the site of the nucleic acid sequence. The complex can be further reacted with a compd. to accentuate the coloration and/or with a decoloration soln. contg. Na₂S₂O₃ and (NH₄)₂S₂O₃. A membrane-immobilized and alk. phosphatase-labeled nucleic acid was reacted with color developing soln. contg. pH 9.5 Tris-acetate 50, Mg acetate 10, cysteamine phosphate 5, and aurothioglucose 3 mM for 15 min - 2 h. HClO₄ or AgNO₃ was used to enhance the visualization, by changing the color from lemon yellow/gold yellow to chestnut brown. By this method, it is possible to detect 1 pg DNA in dot blot hybridization and visualize single genomic DNA sequence after Southern transfer.
 IT 53-57-6, NADPH 5746-40-7, Cysteamine-S-phosphate 7439-92-1, Lead, biological studies 7439-97-6, Mercury, biological studies 7440-06-4, Platinum, biological studies 7440-22-4, Silver, biological studies 12192-57-3, Aurothioglucose 16903-35-8 27025-41-8, Oxidized glutathione 38654-99-8 61-19-8, Adenosine 5'-monophosphate, biological studies 142-72-3, Magnesium acetate 7440-57-5, Gold, biological studies
 RL: ANST (Analytical study)
 (in immobilized nucleic acid staining)
 L3 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2002 ACS
 AN 1984:546793 CAPLUS
 DN 101:146793
 TI Poly(ADP-ribose) synthetase
 AU Ueda, Kunihiro; Zhang, Jingyuan; Hayaishi, Osamu
 CS Fac. Med., Kyoto Univ., Kyoto, 606, Japan
 SO Methods Enzymol. (1984), 106(Posttransl. Modif., Part A), 500-4
 CODEN: MENZAU; ISSN: 0076-6879
 DT Journal
 LA English
 AB A method for the prepn. of immobilized poly(ADP-ribose) synthetase is presented. DNA is required for the immobilized enzyme activity. Histone H1 inhibited the automodification of the immobilized enzyme in the presence of Mg²⁺, and slightly stimulated it in the absence of Mg²⁺. The product polymer synthesized on the immobilized enzyme had branches.
 IT 9055-67-8
 RL: BIOL (Biological study)
 (immobilized, DNA and magnesium and histone H1 effect on)
 L3 ANSWER 13 OF 15 MEDLINE DUPLICATE 4
 AN 77045491 MEDLINE
 DN 77045491 PubMed ID: 990263
 TI Poly(adenylic acid) synthesis in isolated rat liver mitochondria.
 AU Rose K M; Jacob S T
 SO BIOCHEMISTRY, (1976 Nov 16) 15 (23) 5046-52.

Journal code: 0370623. ISSN: 0006-2960.

CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 197701
ED Entered STN: 19900313
Last Updated on STN: 19970203
Entered Medline: 19770129

AB Purified rat liver mitochondria were shown to synthesize poly(adenylic acid) (poly(A)) in vitro. Detection of the poly(A) synthesizing activity was facilitated by addition of NaF to the reaction was shown to be poly(A) by its insensitivity to digestion with pancreatic RNase and RNase T1, its degradation by venom phosphodiesterase and its retention on poly (uridylic acid) 20-23 AMP units and it was covalently attached to the endogenous RNA in the mitochondria. Poly(A) synthesis required ATP and a divalent ion and was maximally active in the pH range of 7-8. The reaction was inhibited by atracyloside, cordycepin triphosphate, Rose Bengal, rifamycin derivative AF/103, sodium pyrophosphate, and N-ethylmaleimide. These studies indicate that the mitochondrial poly(A) polymerase previously described in our laboratory (Jacob, S.T., Rose, K.M., and Morris, H.P. (1974), Biochim. Biophys. Acta 361, 312-320) is involved in the posttranscriptional addition of poly(A) sequence to mitochondrial RNA.

AB . . . T1, its degradation by venom phosphodiesterase and its retention on poly (uridylic acid) 20-23 AMP units and it was covalently attached to the endogenous RNA in the mitochondria. Poly(A) synthesis required ATP and a divalent ion and was maximally active in the pH range of 7-8. The reaction was inhibited by atracyloside, cordycepin triphosphate, Rose Bengal, . . .

L3 ANSWER 14 OF 15 MEDLINE
AN 76053079 MEDLINE
DN 76053079 PubMed ID: 1059099
TI Chromatin and DNA synthesis associated with nuclear membrane in germinating cotton.
AU Clay W F; Katterman F R; Bartels P G
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1975 Aug) 72 (8) 3134-8.
Journal code: 7505876. ISSN: 0027-8424.

CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 197601
ED Entered STN: 19900313
Last Updated on STN: 19900313
Entered Medline: 19760116

AB The synthesis of nuclear DNA and possible attachment sites of chromatin in the cells of cotton (*Gossypium barbadense*) radicles during germination was investigated. Biochemical analysis of nuclear membrane fragments or Sarkosyl-magnesium-membrane complexes indicates that the DNA, including newly replicated DNA, is attached to the nuclear membranes during periods of active synthesis. Electron micrographs of nuclear membrane fragments indicate a physical association between chromatin fibers and the membranes. The attachment site appears to be proteinaceous, since the chromatin is released by protein degradative enzymes as evidenced by biochemical techniques and electron microscopic observations. Short-term labeling results in incorporation into a membrane-associated product indistinguishable from the bulk of nuclear DNA. DNA polymerase activity is also associated with nuclear membrane preparations in which [³H]thymidine triphosphate is incorporated into an acid-insoluble, DNase-sensitive product.

AB . . . chromatin in the cells of cotton (*Gossypium barbadense*) radicles

during germination was investigated. Biochemical analysis of nuclear membrane fragments or Sarkosyl-**magnesium**-membrane complexes indicates that the **DNA**, including newly replicated **DNA**, is **attached** to the nuclear membranes during periods of active synthesis. Electron micrographs of nuclear membrane fragments indicate a physical association between. . .

L3 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AN 1974:95573 BIOSIS

DN BR10:95573

TI STRUCTURAL STUDIES OF BDELLOVIBRIO-BACTERIOVORUS STRAIN 109 HOST INTERACTION WHEN PENETRATION IS PREVENTED.

AU ABRAM D; CASTRO E MELO J

SO Abstr. Annu. Meet. Am. Soc. Microbiol., (1974) 74, 63.

CODEN: ASMACK. ISSN: 0094-8519.

DT Conference

FS BR; OLD

LA Unavailable

IT Miscellaneous Descriptors

ABSTRACT ESCHERICHIA-COLI ATTACHMENT DAMAGE DIVALENT CATION DEPLETED HOST PROTEIN RNA SYNTHESIS INHIBITORS SODIUM POTASSIUM LITHIUM CALCIUM MAGNESIUM

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